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(71) Applicants *(for all designated States except US)*: YEDA RESEARCH AND DEVELOPMENT CO., LTD. [IL/IL]; P.O. Box 95, 76100 Rehovot (IL). RAMOT UNIVERSITY AUTHORITY FOR APPLIED RESEARCH AND INDUSTRIAL DEVELOPMENT LTD. [IL/IL]; 32 Haim Levanon Street, 69975 Ramat Aviv (IL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: YAYON, Avner [IL/IL]; 104, 76834 Moshav Sitiria (IL). NEVO, Zvi [IL/IL]; 11 Yair Stern Street, Neve Amirim, 46425 Herzlia (IL).

(74) Agent: GALLILY, Tamar; Reinhold Cohn & Partners, P.O. Box 4060, 61040 Tel-Aviv (IL).

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(54) Title: FGFR3 AS A MARKER FOR MESENCHYMAL SKELETAL PROGENITOR CELLS

(57) Abstract

The present invention concerns fibroblast growth factor receptor 3 (FGFR3) as a novel marker for mesenchymal skeletal progenitor cells. By utilizing this novel marker it was possible both to identify and locate mesenchymal skeletal progenitor cells in a tissue, as well as to obtain a substantially pure culture of such cells. The pure culture of the mesenchymal skeletal progenitor cells may be used, optionally after various manipulations *ex vivo*, as an active ingredient in pharmaceutical compositions or implants for the purpose of bone and/or cartilage repair. FGFR3 may also be used as a marker for the identification and the localization of cartilage- and bone-derived tumors. Agents capable of binding to FGFR3 may also be used for targeting cytotoxic agents to cartilage- and bone-derived tumors.

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FGFR3 As a Marker For Mesenchymal Skeletal Progenitor Cells

FIELD OF THE INVENTION

The present invention concerns a method for identifying mesenchymal skeletal progenitor cells by identification of cells which feature on their surface fibroblast growth factor receptor 3 (FGFR3).

The present invention further concerns a method for obtaining mesenchymal skeletal progenitor cells by utilizing FGFR3–binding agents. The invention still further concerns a substantially pure culture of mesenchymal skeletal progenitor cells as well as pharmaceutical compositions and implants comprising said mesenchymal skeletal progenitor cells.

By another aspect the invention concerns a method for identification of cartilage–bony tumor and pharmaceutical compositions for the treatment of cartilage–bony tumor.

BACKGROUND OF THE INVENTION

Skeletal growth depends both on proper function of the tissue cellular elements – the chondrocytes, and their cell membrane receptors in the cartilaginous growth centers of the long bones, as well as on the normalcy and levels of circulating and local hormones and growth factors. Growth disorders are therefore classified into two distinct categories (a) failures in a circulating factor, and (b) failures in the target cartilaginous tissue.

The course of normal differentiation begins with mesenchymal stem cells which differentiate to skeletal progenitor cells which can either differentiate to precartilaginous stem cells, which eventually form the cartilage, or to preosteogenic stem cells which eventually form the bone.

In attempts to trace the mesenchymal stem cells supporting growth and their routes of migration in normals and in the family of growth disorders, there are difficulties, including the lack of proper markers for these specific mesenchymal stem cells. For example, spotty and incomplete information is available regarding the original location and the routes of migration of the growth plate stem cells, supporting the longitudinal and the transverse growth. A long lasting dispute of over a hundred years, which may be called *"Ranvier versus La Cro2ix"* is still perpetuating. In 1889 Ranvier stated *"Cells forming the periosteal bone, originate from the cells of the growth plate"*, while in 1951 La Croix declared *"Appositional growth occurs from cells of the peri-chondral periphery"*. Ranvier's theory gained support at the early seventies from Rigal, Hert, J. (*Acta Anat (Bazel)* 82:420–436 (1972)) and others, and in the nineties by Langenskiold *et al.* (Acta. Orthop. Scand., 64:683–687 (1993)), suggesting that cells from the germinal layer migrate to the borderline of the bone groove, serving as the source for both longitudinal and transverse bone growth.

A full understanding of the various types of cartilage cells and the factors that effect mesenchymal differentiation, however, has been hampered due to failure to locate the original location of the primary reservoir of these cells and thus the limitations of *in vitro* cell culture. One difficulty has been the lack of specific phenotypic markers to follow successive differentiation events. Type II collagen secretion is considered a major early marker of chondrocyte differentiation,

while the synthesis of alkaline phosphatase is an early marker of osteoblast differentiation. Mature osteoblasts also produce osteopontin, osteonectin, and osteocalcin, three extracellular matrix proteins deposited together with type I collagen into mineralized bone matrix. Unfortunately, only a few differentiation markers have been identified, and several of these, such as alkaline phosphatase, osteopontin, and osteonectin, are not specific for osteogenic differentiation, while others, such as osteocalcin, are rarely expressed *in vitro*. In addition, mesenchymal cell lines and primary cultures of differentiating chondrocytes and osteoblasts display a variable phenotype and are often a mixture of cell types at different stages of differentiation (Eriebacher, A. *et al*, *Cell* 80:371-378, (1995); Yamaguchi, T.P. and Rossant, J., *Current Opinion in Genetics and Development* 5:485-491 (1995)).

Thus it would have been highly desirable to develop a marker capable of locating precisely the site and source of stem cells supporting and contributing to both longitudinal and transverse growth and for bone repair both for better understanding of the mechanism of mesenchymal development in normal and pathological conditions, as well as for the purpose of obtaining a substantially pure culture of mesenchymal skeletal progenitor cells for therapeutical purposes.

SUMMARY OF THE INVENTION

The present invention is based on the surprising finding that fibroblast growth factor receptor 3 (FGFR3) serves as a marker for mesenchymal skeletal progenitor cells. The present invention is further based on the surprising finding that the anatomical location of mesenchymal skeletal progenitor cells is in the perichondrium in the La Croix groove.

The term *"mesenchymal skeletal progenitor cells"* will be used in the following to denote the following types of cells: (a) mesenchymal stem cells which are able to differentiate to skeletal progenitor cells, (b) skeletal progenitor cells, (c) precartilaginous stem cells, and (d) preosteogenic stem cells or a combination of two or more of the above cell types. The mesenchymal skeletal progenitor cells all share the property of contribution to the growth of bone and/or cartilage, show enhanced proliferation properties as compared to other types of cartilage and bone

derived cells and also a tendency to migrate in the presence of suitable chemotactic agents such as fibroblast growth factor 9.

These mesenchymal skeletal progenitor cells, in early stages of embryonal and neonatal life, support the growth of both articular and physis growth-plate cartilages. However, quite early in life, a few months post-birth, the connection of these stem cells to the articular zone is abolished leading to the poor self-wound healing of articular cartilage. Such mesenchymal skeletal progenitor cells continue to maintain the cell source for the longitudinal and latitudinal (transverse) growth of long bones, until the closure of the physis (at the age of 18-22 years), and continue to provide the stem cell reservoir of the periosteum, involved in the callous of bone fractures all through life. In adult life, especially at advanced ages, a technique for tracing undifferentiated cell source with a potential to establish proliferating chondrocytes has previously failed due to the scarcity of such a cell source and the inadequatability of markers for such undifferentiated cells.

By using the discovery on which the present invention is based, namely that FGFR3 is a marker for mesenchymal skeletal progenitor cells, it was possible to develop a method for identification of mesenchymal skeletal progenitor cells by identifying those cells which feature FGFR3 on their surface. Such a method may be important for tracing mesenchymal skeletal progenitor cells for example for better understanding of pathological conditions of growth arrest involving FGFR3 receptors for example those leading to genetic dwarfism-achondroplasia or persistent expression in multiple hereditary exostosis and reexpression in primary osteoarthritic osteophytes.

Thus the present invention provides a method for identifying mesenchymal skeletal progenitor cells comprising:

(a) applying a fibroblast growth factor receptor 3 (FGFR3) binding agent to assayed cells or tissue under conditions allowing ligand-receptor binding;

(b) determining which cells bound said FGFR3 binding agent, said cells being mesenchymal skeletal progenitor cells.

The FGFR3 binding agent which may be an antibody or fibroblast growth factor 9 (FGF9) should be labeled and applied to the assayed tissue, for

example to tissue of the joint. Those regions which are labeled serve as a source for mesenchymal skeletal progenitor cells.

Preferably, the source for the mesenchymal skeletal progenitor cells is the perichondrium at the region of La Croix, and the region which meets the synovial membrane and the periosteum.

The method of the present invention may be used to identify and locate mesenchymal skeletal progenitor cells in various tissues such as at the joints for various purposes, for example for obtaining a culture of mesenchymal skeletal progenitor cells, proliferating them *in vitro* and then reintroducing them to the body in order to encourage cartilage and bone growth. Alternatively, identifying these cells enables their removal from the tissue site in order to eliminate excess activity of such stem cells in various diseases and disorders characterized by over-activity of undifferentiated mesenchymal skeletal progenitor cells. Furthermore, by locating the regions of the FGFR3 carrying cells, it is possible to manipulate such cells *in situ* by administering to the exact location of these cells various modulating agents. Such agents may be agents capable of stabilizing the FGFR3 and thus maintaining for longer periods of time the undifferentiated proliferating state of these cells, an example being FGF9. Alternatively, the agent may cause premature differentiation of FGFR3 carrying cells, an example being an FGF9 antagonist.

By using the fact that FGFR3 is a marker of mesenchymal skeletal progenitor cells, it was possible for the first time to obtain a substantially pure culture of such cells from a non-embryonic source. According to the method of the present invention, by using the FGFR3 as a marker, it was found that the mesenchymal skeletal progenitor cells are located in the perichondrium ring (region of La Croix) present in the periphery of the growth plates. The poor self-wound healing of articular cartilage late in life may be explained on the basis of disconnection of these articular zones from the source of their potential stem cells at the perichondrial La Croix region which occurs at the cessation of growth.

Thus, the present invention enables not only localization of mesenchymal skeletal progenitor cells, but also obtaining for the first time a substantially pure culture of large amounts of such cells. The term *"substantially pure culture"* denotes a culture composed essentially of one or more of the four cell

types covered by the term *"mesenchymal skeletal progenitor cells"* as defined above.

The present invention thus concerns a method for obtaining large amounts of mesenchymal skeletal progenitor cells from various sources, as will be explained hereinbelow. The mesenchymal skeletal progenitor cells may be identified, and separated from the other cells in the source, by utilizing either specific antibodies against FGFR3, or by using a specific ligand for this receptor such as the FGF9 ligand.

The method for obtaining a substantially pure culture of mesenchymal skeletal progenitor cells comprises:

(c) applying an FGFR3-binding agent to a cell source containing mesenchymal skeletal progenitor cells; and

(d) separating from said source only cells which are bound FGFR3, said cells providing a substantially pure culture of mesenchymal skeletal progenitor cells.

Separation may be carried out surgically, for example by picking up with a scalpel only those regions which are bound to an FGFR3 labeled binding agent, or may be carried out by utilizing various cell separation systems which are able to separate individual cells bearing a specific label (the FGFR3 binding agents) from an unlabeled population of cells in the source.

Suitable sources for obtaining such mesenchymal skeletal progenitor cells is an autogenic source available from arthroscopic or bone marrow biopsies. The biopsy source may be non-proliferating chondrocytes or dedifferentiated fibroblast-like cells. The cell source may also be obtained from regions of the perichondrium, synovial membrane or periosteum or the location in which these regions meet. Only by utilizing a specific marker it is possible to isolate mesenchymal skeletal progenitor cells from these sources due to the scarcity of these cells in the tissue. Alternatively the cell source may also be embryonic.

Mesenchymal skeletal progenitor cells obtained from these sources may be induced to proliferate *ex vivo* in the presence of suitable growth factors and heparin and then reintroduced into the body either in the form of pharmaceutical composition within a medium suitable for maintaining the viability of chondrocytes, or introduced to the desired site in the form of an implant, wherein the mesenchymal skeletal progenitor cells are present inside a growth-permissive gluey milieu.

It is preferable that both the pharmaceutical composition and the implant contain also suitable fibroblast growth factor, preferably fibroblast growth factor 9, in order to stimulate the activity of the FGFR3 present on those mesenchymal skeletal progenitor cells.

The pharmaceutical compositions or the implant of the invention may be used for the purpose of repair and regeneration of defective articular cartilage, for treatment of achondroplastic patients, for treatment of patients suffering from other growth disturbances and for treatment of physical injuries with poor predicted rate of cartilage and bone growth. The pharmaceutical composition or the implant of the invention may be used as interventions for manipulating the rate of growth within growth plates in order to increase the growth rate and/or prevent premature differentiation; or may be used for direct injection into the nucleus pulposus of the fine vertebrae in order to enhance the healing of spine injuries. If desired, the autologous mesenchymal skeletal progenitor cells may be altered, *ex vivo* by molecular engineering to express desirable traits prior to introduction into the desired site. Examples of genetic manipulations are those directed to over-expression of wild type FGFR3 in order to replace a mutant defective receptor, or the expression of a dominant negative mutant FGFR3 in order to suppress the activity of a wild type receptor, for example, in the cases of various types of tumors and the like.

In practice, the method of the invention comprises embedding the mesenchymal skeletal progenitor cells in a viscous growth-permissive milieu, usually based on hyaluronic acid, forming a composite semi-solid implant. The implant is transferred to the target site of growth, for example the articular lesion site, either under open joint surgery or by an arthroscopic device, filling the lumen of the injury to the articular surface. A thin permeable film is formed by a spraying device, closing the defect and ensuring the anchorage and maintenance of the implant in its authentic place.

By another aspect, the present invention is based on the finding that FGFR3 is also present on cartilagineous-bony tumors, for example, benign tumors (e.g. exostosis and osteophytes) and thus may serve both as an indicator of the presence of such a tumor as well as a marker for the precise localization of such

tumors. Therefore, the present invention further comprises a method for detection of cartilaginous–bony tumors in a tissue or a sample comprising:

(i) contacting the assayed tissue or sample with an FGFR3 binding agent;

(ii) detecting the presence of cells which bound FGFR3 binding agent a positive detection indicating the presence of a cartilaginous–bony tumor in the assayed tissue or sample.

The detection may be carried out by using suitable labeled antibodies against FGFR3, or by use of specific labeled ligands for FGFR3, such as FGF9. By applying said labeled FGFR3 binding agent to a tissue *in vivo* it is possible not only to determine whether a tumor is present in the tissue, but also to precisely localize the tumor which may help in surgical removal thereof.

The fact that the FGFR3 is present on cartilaginous–bony tumor cells, may also serve to target cytotoxic agents specifically to the site of the tumor, by attaching to a specific ligand to FGFR3 such as FGF9, or a specific antibody against FGF9, a suitable cytotoxic moiety. Thus the present invention is further directed to pharmaceutical compositions for the treatment of cartilaginous–bony tumors comprising an FGF3 binding agent attached to a cytotoxic moiety, as well as to a method for the treatment of such tumors by administering to a subject a therapeutically effective amount of FGFR3 attached to a cytotoxic moiety.

Cytotoxic agents are well known in the art and this term, within the context of the present invention, refers to any agent capable of destroying cartilage- and bone-derived tumor cells. Examples of such agents are, for example methotrexate, doxombicin, cyclophosphamides. etc.

Treatment of cancer may also be carried out by inducing differentiation of FGFR3 carrying cells. This may be carried out for example by introduction of FGFR3 differentiation inducing agents to regions labeled by a FGFR3–binding agent. Examples of differentiation inducing agents are FGF9 antagonists or antibodies against FGF9.

The treatment may also be carried out by introducing to the tumor a dominant negative detective FGFR3 (for example, by genetic engineering) which attenuates the activity of the wild type FGFR3.

In the following the invention will be illustrated with reference to some non-limiting drawings and examples.

DETAILED DESCRIPTION OF THE DRAWINGS

Fig. 1 – Histological staining of epiphyseal section of guinea pig by antibodies against FGFR3. Photo-micrographs 1-5 represent sections through the epiphyses of young adult guinea pig.

1. Sagittal section stained with the histological dye of Masson's trichrome (magnification x 6) which is a specific staining for connective tissue elements such as collagen and protoglycans.
2. Sagittal section stained by immunohistochemical staining with antibodies against FGFR3 (x 400). 3, 4 & 5. Axial sections.
3. Immunohistochemical staining for the antibody agent FGFR3 (x 100);
4. Masson's trichrome (x 25); and
5. Immunohistochemical staining for the antibody agent FGFR3 (x 400).

Fig. 2 consists of photomicrographs 6-11 representing sagittal sections through the epiphyses of 17 days old chick embryos of long bones.

Photomicrographs 6, 10 & 11 are stained by the immunohistochemical staining with antibodies against FGFR3 and 8 is stained by Alcian blue pH 2.5 specific for protoglycans (note lack of staining in certain areas).

The photomicrographs are magnified as follows: 6 (x 25); 7 (x 40); 9 (x 100); 10 (x 400); and 11 (x 100).

7 & 9 are stained by Masson's trichrome.

Fig. 3 shows the femur growth in adolescent rats in which the prechondrian ring surrounding the physis was removed (STRIP); in rats which underwent exposure of perichondrium without its removal (SHAM); and in rats which did not undergo any operation (CNTL); and

Fig. 4 shows the number of days until colony formation of cells, grown *in vitro*, obtained from articular cartilage, epiphysis, physis and perichondrium.

DETAILED DESCRIPTION OF THE INVENTION

Materials and Methods

(a) Primary Chondrocyte Culture:

Epiphysis of long bones (femur and tibia) were obtained from 11 days old chicken embryos. After dissection, tissue segments were treated with trypsin in Tyrod's solution and mechanically disrupted until free cell suspension was obtained. Cells were then plated in high concentration ($5x10^6$).

(b) PCR screening of primary chondrocyte cultures:

When confluence was reached, cells were collected and lysed by RNA purification kit (tri-reagent) (Molecular Research Center, Cincinnati, Ohio). RNA from cells was phenol extracted, isopropanol precipitated, resuspended in water, and assayed by measuring its optic density. After obtaining clean RNA (O.D. 260/280nm.>1.5), cDNA using reverse transcriptase reaction was made and screened for fibroblast growth factors (FGFs). The polymerase chain reaction (PCR) technique was used, employing oligonucleotide pairs for both FGFR3 and FGF9. Denaturation was at 94°C, annealing at 52-65°C, and elongation at 72°C, repeated for 35 cycles.

(c) Radiolabeling of FGF9:

Recombinant mouse FGF9 was prepared as previously described (Hecht, D. *et al*, Growth factors **12**, 223-233 (1995)) labeled with $Na^{125}I$ (0.5mCi) using the Chloramine-T method and separated from free iodine on a heparin-sepharose column. The range of specific activity was 0.5-2 x 105 cpm/ng.

(d) Immunohistochemistry:

Decalcified bones were embedded in liquid paraffin after fixation by formalin and picric acid. Paraffin blocks were cut and prepared for immunohistochemistry using a standard protocol. Staining of slides was done with ascending titer of anti-FGFR3 antibody.

(e) *In situ* hybridization:

T7 (antisense probe) and T3 (sense probe) were made from recombinant FGF9 and FGFR3 containing plasmid (Bluescript–Stratagen), using S–35 labeled uridine residues. Mouse embryos aged 10.5 to 18.5 days post–conception were fixed in paraformaldehyde, dehydrated in ascending concentrations of ethanol, and embedded in liquid paraffin. Sections were cut and prepared and hybridized with a proper RNA probe by standard methods.

Example 1

Histochemical staining by antibodies against FGFR3

As can be seen in Fig. 1, regions which were stained with antibodies against FGFR3 did not correspond to regions stained by Masson's trichrome which is an accepted cartilage stain. These findings indicate that FGFR3 bearing cells are not located in the cartilage itself but rather in the perichondrium in the region known as La Croix groove.

Example 2

Stripping of the ring of La Croix in adolescent rats

10 rats were included in each of three groups. Group 1 served a s a control group (CNTL). Rats were anesthetized but no operation was performed. Group 2 (SHAM) served as a sham operation group and underwent anesthesia and dissection of soft tissues exposing the perichondrium. Group 3 (STRIP) underwent stripping of the perichondrial ring surrounding the physis under loop magnification which allowed dissection of the soft tissues only without any damage to the physis itself. 4 weeks later average femur length was measured in rats and the results are shown in Fig. 3. The contra–lateral limb was similar in length in operation to the control limbs (data not shown). The sham operated limbs demonstrated a tendency for increase in limb length which did not reach statistical significance. The stripped limbs demonstrated a growth arrest of the limb. These results indicated that removal of regions which were stained by FGFR3–antibodies causes arrest in limb length indicating the involvement of such regions in normal growth.

Example 3

***In vitro* growth of cells obtained from the ring of La Croix**

Perichondrial tissue from the La Croix region removed from the above rats was placed in a culture dish in a suitable growth medium and the period until colony formation was determined. In comparison, tissue obtained from various locations of the distal femur (articular cartilage, epiphysis (bone), physis (cartilage)) was cultured under the same conditions and the period until colony formation was also determined.

As can be seen in Fig. 4, tissue removed from the perichondrium demonstrated an ability to rapidly form cell colonies after about 3 days in culture, while tissue removed from other regions formed cultures only after more than ten days from implantation. These results again indicate that cells obtained from the region stained with FGFR3-antibodies grow more rapidly than cells obtained from other regions of the bone which do not feature FGFR3.

Example 4

Presence of FGFR3 in exostosis

Antibodies against FGFR3 were applied to tissue obtained from exostosis benign tumor. The antibodies stained cells in the fibrotic tissue and essentially did not stain cells of the cartilage (data not shown). These findings indicate that FGFR3 is present in cartilaginous-bony derived benign tumor (exostosis) so that FGFR3 binding agents (such as antibodies) may be used to identify such tumors as well as to target cytotoxic agents thereto. This finding also leads to the treatment of such tumors by agents which cause disappearance of FGFR3 (for example antagonist of FGF9) and thus lead to differentiation.

CLAIMS:

1. A method for identifying mesenchymal skeletal progenitor cells comprising:

(a) applying a fibroblast growth factor receptor 3 (FGFR3) binding agent to assayed cells or tissue under conditions allowing ligand-receptor binding;

(b) determining which cells bound said FGFR3 binding agent, said cells being mesenchymal skeletal progenitor cells.

2. A method according to Claim 1, wherein the FGFR3-binding agents are antibodies specific for FGFR3.

3. A method according to Claim 1, wherein the FGFR3-binding agent is fibroblast growth factor 9 (FGF9).

4. A method for obtaining a substantially pure culture of mesenchymal skeletal progenitor cells comprising:

(a) applying FGFR3-binding agent to a cell source containing mesenchymal skeletal progenitor cells; and

(b) separating from said source cells which are bound FGFR3, said cells providing a substantially pure culture of mesenchymal skeletal progenitor cells.

5. A method according to Claim 4, wherein the cell source is autogeneic being non-proliferating chondrocytes, dedifferentiated fibroblast-like cells, cells obtained from the perichondrium, synovial membrane, periosteum, or the cell source is embryonic.

6. A substantially pure culture of mesenchymal skeletal progenitor cells.

7. Mesenchymal skeletal progenitor cells obtained by the method of Claim 5.

8. A pharmaceutical composition for the repair of bone and cartilage comprising a medium suitable for maintaining the viability of chondrocytes and mesenchymal skeletal progenitor cells of Claim 6 or 7.

9. A pharmaceutical composition according to Claim 8 further comprising a member of the FGF family.

10. A pharmaceutical composition according to Claim 9, comprising FGF9.

11. An implant suitable for bone or cartilage implantation comprising a growth permissive gluey milieu and mesenchymal skeletal progenitor cells of Claim 6 or 7.

12. An implant according to Claim 9 further comprising a member of the FGF family.

13. An implant according to Claim 12, comprising FGF9.

14. A method of treating achondroplastic patients, patients suffering from other growth disturbances and physeal injuries with poor predicted fate of cartilage and bone growth comprising: administering to or implanting in the site of desired growth or repair the pharmaceutical composition of Claims 6 to 8 or the implant of Claim 9 or 10, respectively.

15. A method according to Claim 14, wherein the administered cells are autogeneic.

16. A method according to Claim 14, wherein the cells are allogeneic.

17. A method for detection of cartilaginous–bony tumors in a tissue or a sample comprising:

(i) contacting the assayed tissue or sample with an FGFR3 binding agent;

(ii) detecting the presence of cells which bound FGFR3 binding agent a positive detection indicating the presence of a cartilaginous–bony tumor in the assayed tissue or sample.

18. A method according to Claim 17, wherein the cartilaginous–bony tumors is a benign tumor selected from the group consisting of: exostosis and osteophytes.

19. A pharmaceutical composition for the treatment of cartilaginous–bony tumors comprising a pharmaceutically acceptable carrier and as an active ingredient an FGFR3 binding agent attached to a cytotoxic moiety.

20. A pharmaceutical composition according to Claim 19, wherein the FGFR3 binding agent is FGF9.

21. A pharmaceutical composition according to Claim 19, wherein the FGFR3 binding agent is an antibody against FGFR3.

22. A method for specifically destroying cartilaginous–bony tumor cells, comprising administering to a subject in need of such treatment a therapeutically effective amount of a FGFR binding agent conjugated to a cytotoxic moiety.

23. A method according to Claim 22, wherein the FGFR3 binding agent is FGF9.

24. A method according to Claim 22, wherein the FGFR3 binding agent is an antibody against FGFR3.

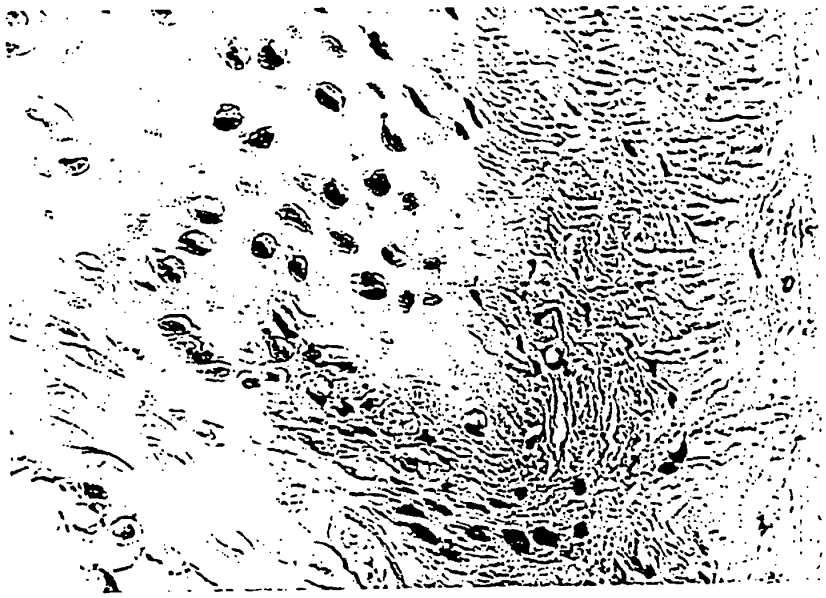

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Fig. 1

Fig. 1 (cont.1)

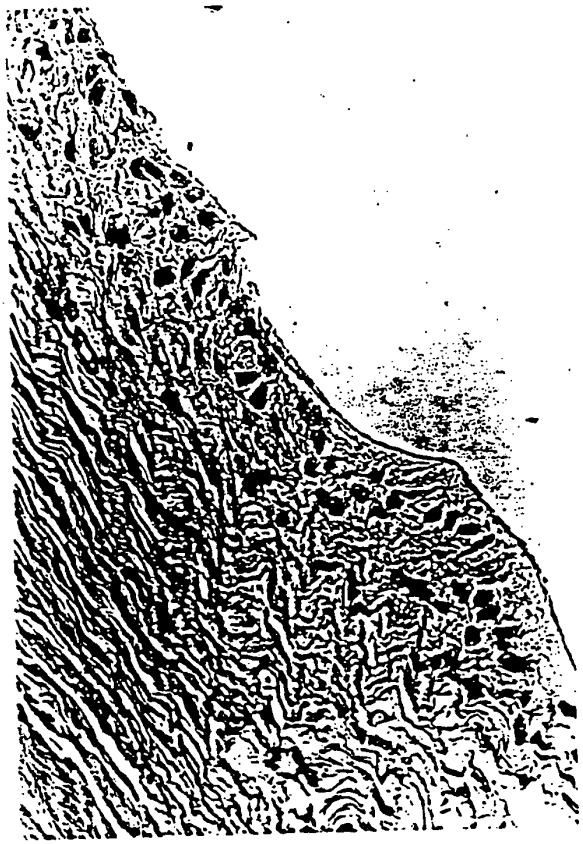

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Fig. 1 (cont.2)

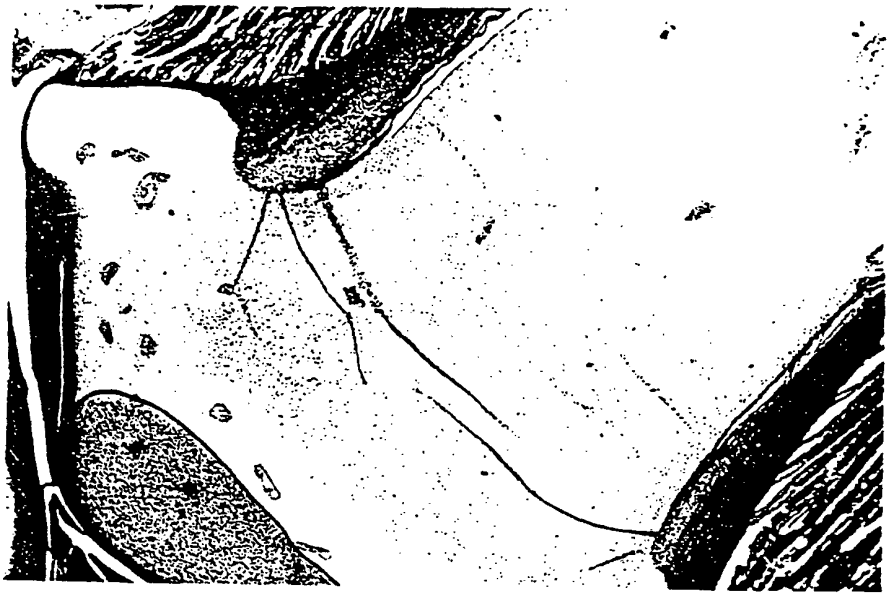

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Fig.2

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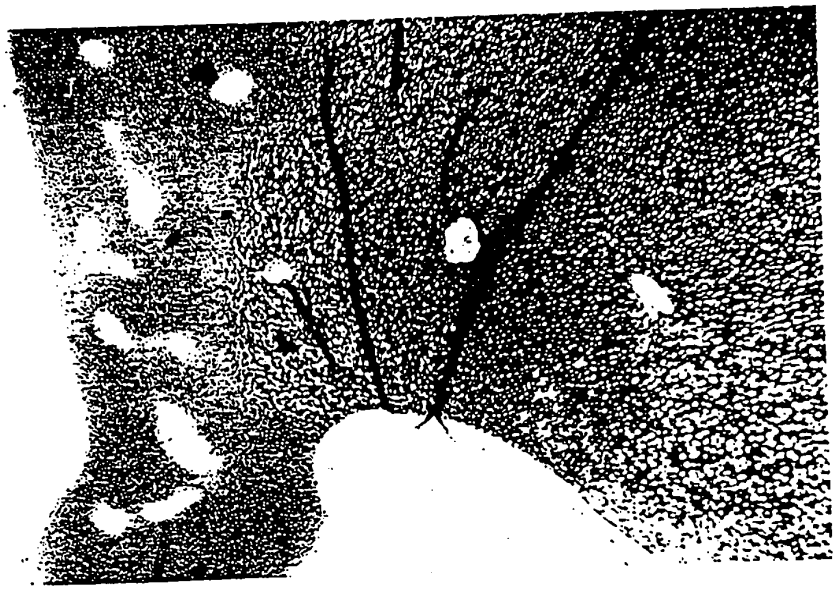

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Fig. 2 (cont.1)

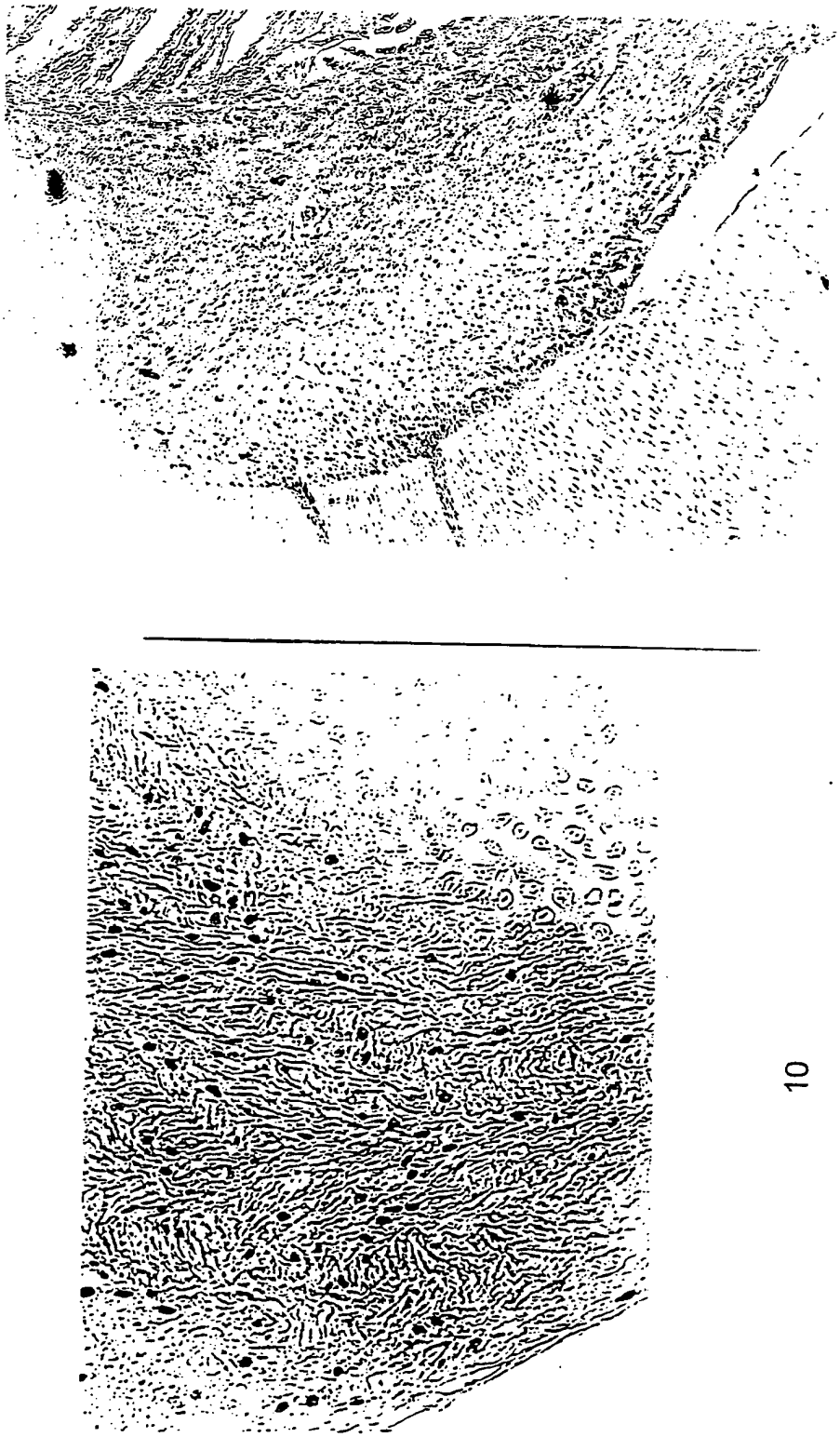

Fig. 2 (cont.2)

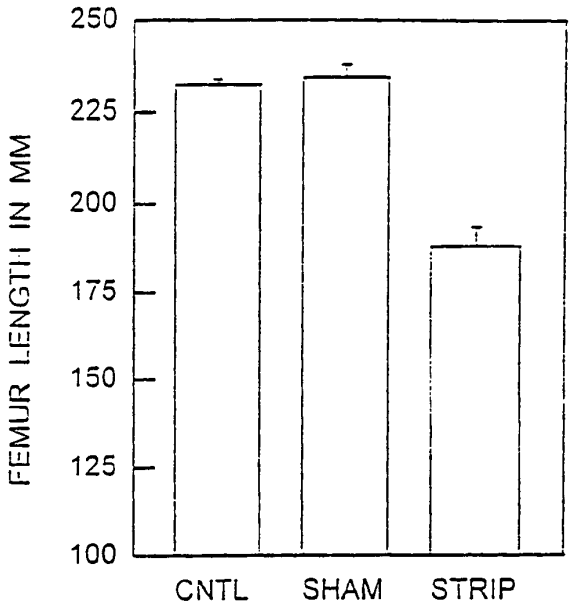

Fig. 3

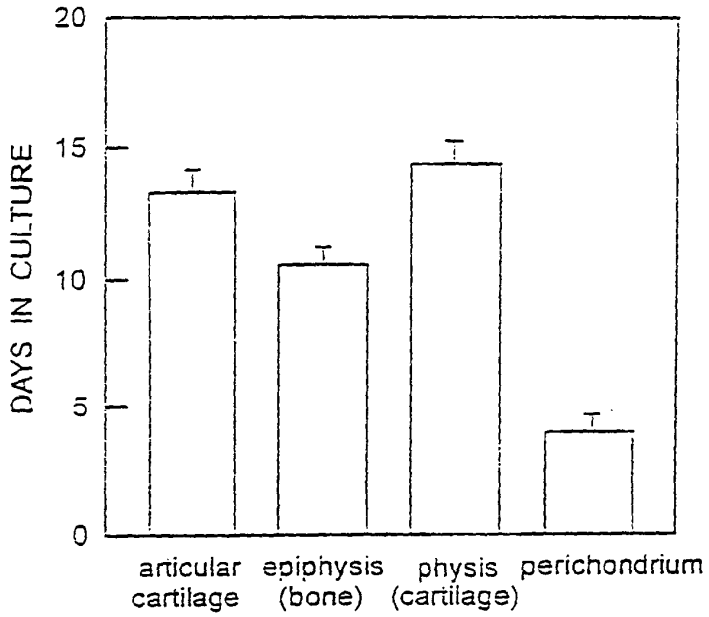

Fig. 4

INTERNATIONAL SEARCH REPORT

Int .tional application No.
PCT/IL96/00010

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 9/22, 39/395; C07K 16/00; C12P 21/08; C12N 5/00; G01N 33/558
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/131.1, 152.1; 435/240.1, 240.2; 436/514; 530/387.1, 388.22, 389.7, 389.7; 604/890.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, MEDLINE, WPID, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROUSSEAU et al. Mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia. Nature. 15 September 1994, Vol. 371, pages 252-254, see entire document.	1-24
Y	SUPERTI-FURGA et al. A glycine 375-to-cysteine substitution in the transmembrane domain of the fibroblast growth factor receptor-3 in a newborn with achondroplasia. Eur. J. Pediatr. March 1995, Vol. 154, pages 215-219, see entire document.	1-24
Y, P	DENG et al. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cell. 22 March 1996, Vol. 84, pages 911-921, see entire document.	1-24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
07 NOVEMBER 1996	19 NOV 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PATRICK NOLAN Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/IL96/00010

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LAUNAY et al. Comparative analysis of the tissue distribution of three fibroblast growth factor receptor mRNAs during amphibian morphogenesis. Differentiation. 1994, Vol. 58, pages 101-111, see entire document.	1-24

INTERNATIONAL SEARCH REPORT

Ir ıational application No.
PCT/IL96/00010

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/131.1, 152.1; 435/240.1, 240.2; 436/514; 530/387.1, 388.22, 389.7, 389.7; 604/890.1